From Embryo to Adult: Hematopoiesis along the *Drosophila* Life Cycle

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Studies on *Drosophila* hematopoiesis have thus far focused on the embryonic and larval origin of hemocytes, the fly blood cells. In this issue of *Developmental Cell*, Ghosh et al. (2015) identify adult hematopoietic hubs containing progenitors that can differentiate into different blood cell types.

Hematopoiesis is a complex cellular process that provides metazoans with blood cells throughout their life cycle. Although the term is often associated with stem cell differentiation in the bone marrow of mammals, hematopoiesis has also been characterized in other organisms such as the fruit fly *Drosophila*. In this insect, hemocytes were thus far thought to be produced in only two waves, one in the embryo and a second one in larvae, with adult hemocytes deriving from larval and embryonic hemocytes. A recent study in this issue of *Developmental Cell* challenges this view as it identifies a hematopoietic site in the dorsal part of the fly abdomen (Ghosh et al., 2015). This, together with other recent studies, suggests a higher-than-expected plasticity in the mechanisms underlying blood cell production in *Drosophila*.

One attractive feature of *Drosophila* hematopoiesis is its apparent simplicity (Honti et al., 2014). First, *Drosophila* as an arthropod has an open circulatory system, and its body cavity is simply filled with a fluid called hemolymph, which is set in motion by a simple contractile "heart" tube. Second, there are only three blood cell types (hemocytes): the plasmatocytes are macrophage-like cells, the crystal cells produce phenoloxidases responsible for melanization, and the lamellocytes are large flat adhesive cells that participate in encapsulation and are induced upon parasitoid wasp infestation. Third, many features in their hematopoiesis are shared among *Drosophila* and mammals, despite some 550 million years of divergence (Evans et al., 2003).

The identification of various hemocyte markers together with the development of new clonal and lineage tracing methods have significantly renewed our understanding of *Drosophila* hematopoiesis. A first step was the demonstration that considerable plasticity still exists in the supposedly stable hemocyte lineages. Indeed, recent studies have revealed that plasmatocytes are able to trans-differentiate into either lamellocytes (Honti et al., 2014) or crystal cells (Leitao and Sucena, 2015). A second major change is that *Drosophila* hematopoiesis is not restricted to the embryonic mesoderm or to the lymph glands, as initially thought, but can take place either in circulation or in periphery in the so-called larval sessile patches. Sessile hemocyte patches are composed of resident hemocytes binding to the subepidermal surface of body wall segments (Lanot et al., 2001; Makhijani et al., 2011; Márkus et al., 2009). Recent studies have shown that sessile hemocytes form a diffuse hematopoietic organ that significantly contributes to the storage, the multiplication, and the differentiation of *Drosophila* hemocytes (Bretscher et al., 2015; Leitao and Sucena, 2015; Makhijani et al., 2011). The importance of these resident hemocyte clusters echoes recent observations in mammals showing the importance of hematopoiesis in peripheral tissues, independent of the bone marrow.

The study by Ghosh et al. (2015) adds a new inflection to our understanding of the *Drosophila* hematopoietic system by highlighting the existence of a diffuse hematopoietic hub in the adult (Ghosh et al., 2015). Ghosh et al. identify four large hemocyte clusters in the dorsal part of the fly abdomen, which they name "adult hematopoietic hubs" (Figure 1). In these clusters, hemocytes are embedded in a network of extracellular matrix components including Laminin A and Pericardin. Using marker analysis and the G-Trace lineage tracing system, the authors provide evidence for the presence of progenitor cells in the hubs that can differentiate into plasmatocytes or crystal cells, which demonstrates that these hemocyte hubs form true hematopoietic organs. Interestingly, the adult progenitor cells originate from the tertiary and quaternary lobes of the larval lymph glands, whose function thus far was unknown.

This is also the first paper to fully characterize a discrete population of crystal cells in adults, a cell type believed to be specific to embryonic and larval stages. It will be interesting in the future to decipher whether those few crystal cells are the sole source of prophenoloxydases in adult flies. Furthermore, since crystal cells and lamellocytes can both differentiate from plasmatocytes, it is now less clear what defines the progenitor cells found in the hubs and whether intermediate stages between pluripotent and fully differentiated hemocytes exist.

Previous studies have shown that hemocyte numbers tend to decrease with age in adult flies (Horn et al., 2014). A temporal analysis by Ghosh et al. shows that plasmatocyte numbers in the hub increase until 5 days post eclosion (dpe), due to both the differentiation from progenitors and the recruitment of circulating hemocytes (Ghosh et al., 2015). Hub plasmatocyte numbers then remain constant until 8 dpe and later decline with age. It was thought that adult plasmatocytes cannot divide, and, indeed, no mitotic activity is detected by these authors in non-infected flies. However, mitotic plasmatocytes, as evidenced by BrdU staining, are observed in young adults after bacterial

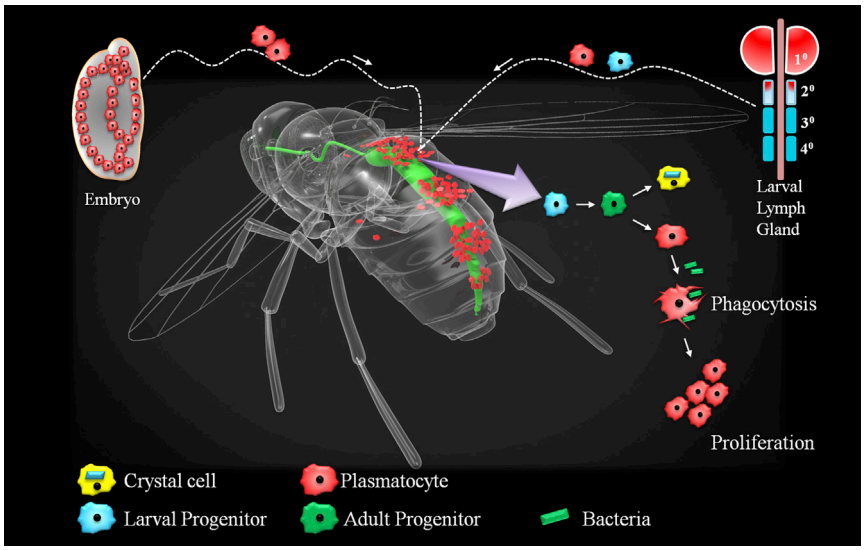

Figure 1. Hematopoietic Hubs in Adult *Drosophila*
Drosophila adult hematopoiesis relies on four clusters located dorsally on the fly abdomen, along the cardiac tube. These clusters house numerous plasmatocytes from embryonic and larval origins, as well as functional crystal cells. But these hubs also harbor adult progenitors, originating from precursors born in tertiary and quaternary lobes of larval lymph gland precursors, and which can differentiate into both new plasmatocytes and crystal cells. Upon immune challenge, quiescent cells re-enter into proliferation to increase the production of plasmatocytes to face pathogen. Image crdit: Saikat Ghosh.

infection, indicating that an immune signal may induce a surge in hemocyte proliferation in the adult. This is comparable to the situation in vertebrates in which an immune response triggers enhanced hematopoiesis in the bone marrow and in peripheral tissues. The search for molecular signals, which modulate hematopoiesis during an immune response, should be an important research axis in the future. Future work should also aim at identifying the signals, hemocyte receptors, and tissue-secreted factors that promote either the homing of hemocytes to hub sites or their recruitment in circulation upon infection or wounding. One of the former is the Nimrod receptor Eater, which is required cell autonomously in plasmatocytes for their homing to the sessile compartment in larvae (Bretscher et al., 2015).

Some might wonder why these adult hematopoietic hubs have only now been characterized. One reason may be that most research on *Drosophila* hematopoiesis has thus far taken a developmental perspective, focusing on embryos and larval lymph glands. Nevertheless, several studies have already noted the presence of resident hemocytes in the dorsal abdomen of flies but did not consider these clusters true hematopoietic sites. How are adult hemocyte hubs related to sessile hemocyte patches in larvae? In larval sessile islets, hemocytes are interconnected by septate junctions, display thin extensions, and are sometimes in contact with neurons or oenocytes (Lanot et al., 2001; Makhijani et al., 2011; Márkus et al., 2009). These features have not yet been described in adult hematopoietic hubs, suggesting a simpler organization. Thus, the biological relevance of sessile hemocytes is not yet fully understood. It would also be interesting to know whether *Drosophila* adult hubs vary across populations, as hemocyte numbers change markedly from one strain to another.

Despite intense studies on the fly immune system over the last few decades, the hemocyte cellular response has not yet received the attention it deserves, largely due to the rather subtle immune phenotypes observed upon knocking down hemocyte functions. Several recent studies revealed a much broader trophic role for hemocytes, as they contribute to the formation of basement membrane, tissue repair, elimination of unfit cells, and lipid homeostasis (Woodcock et al., 2015). Our knowledge of *Drosophila* hematopoiesis should benefit from a better understanding of hemocyte immune and physiological functions. The compartmentalized and plastic features of *Drosophila* hematopoiesis make it an excellent model system to simultaneously investigate basic developmental processes, homeostatic tissue interactions, and rapid adaptation to immune challenges.

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